MICROBIOLOGICAL DETERIORATION SERIES

REPORT NO. 6

15

A FIELD SURVEY OF THE MICROBIOLOGICAL CONTAMINATION PRESENT IN JP-4 FUEL AND 115/145 AVGAS IN A MILITARY FUEL DISTRIBUTION SYSTEM

QUARTERMASTER RESEARCH & ENGINEERING CENTER

PIONEERING RESEARCH DIVISION

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NATICK, MASSACHUSETTS

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Quartermaster Research & Engineering Center, Natick, Mass.
A FIELD SURVEY OF THE MICROBIOLOGICAL CONTAMINATION PRESENT IN JP-4 FUEL AND 115/145 AVGAS IN A MILITARY FUEL DISTRIBUTION SYSTEM by Morris R. Rogers and Arthur M. Kaplan. 40 pp illus (Microbiological Deterioration Series Report No. 6)

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UNCLASSIFIED

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2. Aviation fuels
3. Rusts (Microorganisms)
4. Molds (Organisms)
5. Fungi
6. Microorganisms
7. Bacteria
8. Storage tanks

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Morris R. Rogers Arthur M. Kaplan

Fungicides and Germicides Laboratory

Project Reference:
1K012501A031

June 1963

FOREWORD

This report presents the results of a study to determine the microbial populations indigenous to fuel (JP-4 and 115/145 Avgas) in a typical civilian supplier and military user fuel handling system. Most petroleum studies reported in the literature have obtained their microbiological results with fuel and water samples shipped back to the laboratory for analysis. In contrast, this study utilizes freshly drawn samples cultured on site within 1 hour after sampling in the field. The microbial populations reported herein therefore reflect a more accurate count of the microorganisms present in the system and may permit a more accurate estimate to be made of the relationship existing between poor fuel handling practices and microbial contamination.

CONTENTS

ABSTRACT

Microorganisms have either been implicated in or suspected of contributing to the fouling of liquid hydrocarbon fuels and the corrosion of fuel storage, handling and distribution equipment as well as interference with engine performance. Major problem areas have been with aircraft filter and fuel probe fouling as well as wing tank coating deterioration and aluminum corrosion. To provide information relative to the microbial load in such a system, it became pertinent to determine the nature and numbers of microorganisms found in a "typical" military fuel distribution system. This report presents the findings of a microbiological field survey as conducted on 21 through 25 May 1962 of the fuel distribution system at Pease Air Force Base, New Hampshire, and its civilian supplier, New England Tank Industries, Newington, New Hampshire.

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A FIELD SURVEY OF THE MICROBIOLOGICAL CONTAMINATION PRESENT IN JP-4 FUEL AND 115/145 AVGAS IN A MILITARY FUEL DISTRIBUTION SYSTEM

1. Introduction

The presence of microorganisms in fuel storage water bottoms has caused concern in United States military and civilian aviation operations. Microbial growth is supported by hydrocarbon fuels and microorganisms have been implicated in the production of sludge and slime in fuels. Microbially produced materials may cause malfunctioning of fuel pumps and fuel capacitance gages, clogging of filter separators, deterioration of integral fuel tank coatings, corrosion of integral fuel tanks as well as bulk storage tanks, and emulsification of water in fuel which may cause icing of fuel systems at high altitude (1-4). Although microbial attack on jet fuels has received the widest publicity, it is known that bacteria and fungi also readily grow in gasoline, diesel fuels, lubricating oils, hydraulic fluids, oil emulsions and sundry other hydrocarbons.

The problem of fuel hydrocarbon decomposition also has been noted in areas other than the United States. English fuel technologists reported an explosion in Yorkshire, England, in 1936 resulting from microbial action (presumably methane production) at the bottom of a fuel storage tank (5). In 1952 the Royal Air Force reported trouble with H_2S corrosion of piston aircraft in Egypt (6). Since 1952 there have been other similar cases of contamination of aviation fuel (jet and gasoline) in Egypt, North Africa and Malaya (7). In May, 1960, the Royal Australian Air Force and the Australian Qantas Empire Airways reported the presence of a brown-black slimy growth beneath the kerosene in the integral fuel tanks of Hercules (Lockheed aircraft C130A) and Electra (Lockheed aircraft L 188) aircraft (8). Reports of similar problems in the United States with Electra aircraft operated by National Airlines and Eastern Airlines (8) drew further attention to what appeared to be a major world-wide problem in fuels used for turbine-powered aircraft. Sulfate-reducing bacteria have been reported by the Defence Research Laboratory (Stores), Kanpur, India in 1961 as growing profusely in petrol (gasoline) tanks of aircraft (9).

Studies have shown that many types of bacteria, fungi, and actinomycetes are found in fuel storage tanks. Bakanauskas (1) isolated a total of 71 bacteria consisting of at least three different genera from an unknown number of water bottoms of JP-4 fuel storage tanks located at Lincoln Air Force Base (Nebraska), Schilling Air Force Base (Arkansas), and Davis-Monthan Air Force Base (Arizona). However, no filamentous fungi or strictly anaerobic bacteria were isolated. More recent Air Force studies have shown that fungi also are present in contaminated fuels (3, 11, 15). Klemme and Leonard (4) reported the presence of both fungi and bacteria in JP-5 fuel. DeGray and Killian (10) reported Bacillus and

Pseudomonas as the dominant organisms in water-petroleum fraction interfaces of refinery and bulk terminal storage tanks.

Churchill and Leathen (11) isolated 179 microorganisms from 27 water-jet fuel samples that were held in 1/2-gallon metal containers from 6 to 8 months before the start of the microbiological analyses of these samples. Of the 179 organisms isolated, 75 were fungi and 104 were bacteria. These organisms were further grouped into ten general groups: green fungi, brown fungi, gray fungi, yellow-green fungi, black fungi, miscellaneous fungi, opaque bacteria, mucoid bacteria, transparent bacteria, and chromogenic bacteria. Ten different media were used in these isolations, viz., nutrient agar, Sabouraud's agar, nutrient broth, Sabouraud's broth, and a mineral salts medium. Five special media were also used in an attempt to isolate specific groups of organisms: Beckwith's medium for sulfate-reducing bacteria, Waksman's liquid and solid media for sulfur-oxidizing bacteria, special medium for sulfur-depositing bacteria, Leathen's medium for iron-oxidizing bacteria, and a special medium for iron-depositing bacteria. None of these organisms was isolated except iron-depositing bacteria which were present in one jet fuel-water sample from five of the nine bases examined.

Powelson (12) reported that counts in water phases of samples from storage tanks showed viable bacteria ranged from 90 to as high as 100 million/ml, and mold from less than 100 to 10,000/ml. Sulfate-reducing anaerobes were present, in the range of less than 100/ml to more than 100,000/ml. Fresh and old fuel samples examined included aviation fuels as well as diesel fuel and stove and furnace oils. Powelson did not indicate how much time elapsed between sampling and culturing.

As far as is known, all previous references to the numbers and types of organisms associated with contaminated fuel and interface-water bottom samples have been based on shipped samples. Some of the figures obtained by Powelson (12) may have been made at the site of the storage area; however, this point is not discussed by her except for her statement that fresh and old samples were examined.

No published data exist on the microbial populations of JP-4 other than a listing of the microorganisms isolated.

As in water analysis, it is assumed that an accurate, quantitative plate count of the microbial population of contaminated fuel-water samples cannot be reported for shipped or mailed samples. Bacteria, for example, are usually sensitive to external conditions and quickly respond to slight changes in their environment. Temperature, moisture, pH, and oxygen are important in controlling their numbers and distribution. But the most significant factor is the amount of food supply.

Population changes may occur during shipment, so that there may be little or no relationship between the population of fresh and shipped

samples. The U. S. Air Force - U. S. Army Corps of Engineers' jointly sponsored Project BEARS is currently conducting a study to determine the effects of microbial contamination of JP-4 and 115/145 Avgas fuels on the efficiency of the filter/separator equipment in the Pritchard aircraft refueling system (16). A comparison of microbial counts of the October 1962 fuel and water samples was made between on-site analyses at Kindley Air Force Base, Bermuda, and analyses of samples shipped into the Aeronautical Systems Division, Wright-Patterson Air Force Base, Ohio. Preliminary data show significant differences between on-site analyses at Kindley and those obtained at Wright-Patterson. Decreased microbial counts were noted in the shipped-in samples analyzed at Wright-Patterson (17).

Sharpley (13) states, "There is no halfway measure in describing the sampling of water for microbiological examination. A flat, unequivocal statement can be made: reliable, quantitative microbiological plate counts cannot be obtained on shipped or mailed samples." Other references are cited by Sharpley to substantiate this claim.

2. Purpose of this study

To obtain accurate data on the microbial populations in JP-4 and 115/145 Avgas flowing through a "typical" military fuel distribution system, a microbiological field survey was made to determine:

1. What is the microbial population present in representative samples of JP-4 and 115/145 Avgas fuels and in water samples taken from a fuel distribution system?

2. Is there a change in the microbial population between the time the fuel enters and leaves the distribution system?

3. Are there any points in the system that favor the build-up of microorganisms?

4. Do the filter-coalescers in the system reduce the number of microorganisms found in earlier stages?

3. Fuel storage and distribution system

New England Tank Industries (hereafter called NETI), Newington, N.H., and Pease Air Force Base, N.H., were selected for this field test because of their convenient location. NETI is under contract with the Air Force Logistics Command to supply Pease Air Force Base with all its JP-4 and 115/145 Avgas fuel requirements. Since Pease Air Force Base is a SAC (Strategic Air Command) Base, the fuel volume requirements are very high and consequently fuel turnover is rapid.

Pipeline shipments of JP-4 are made at least three times a week to Pease Air Force Base which is located slightly over 1-1/3 miles from NETI.

JP-4 fuel is supplied to Pease Air Force Base via a 10-inch pipeline. In addition, pipeline shipments of 115/145 Avgas are made at least once a week to Pease Air Force Base along the same route, via an 8-inch pipeline.

NETI is located on the Piscataqua River, which flows into the Maine Harbor. This location permits tankers to deliver fuel directly from refineries in New Jersey and Pennsylvania (or from any other coastal area). The fuel is delivered directly from the tanker to bulk underground storage tanks at NETI. Two 80,000-barrel and two 50,000-barrel below ground tanks are used to store the JP-4 fuel (equivalent to 3,360,000 and 2,100,000 gallons respectively). Two other 50,000-barrel below ground tanks store the 115/145 Avgas.

Fuel leaving the bulk storage tanks at NETI is transported to two 50,000-barrel and one 30,000-barrel above ground intermediate bulk storage tanks located at Pease Air Force Base. Fuel on demand is then diverted through a pump house where it passes through one or more filter separators before entering the hydrant laterals. Finally, just before entering the airplane fuel tank, the fuel is passed through a hose cart which is a liquid-fuel separator or, essentially, a filter-coalescer used to remove the last traces of undissolved water and particulate matter that may remain in the fuel. See Figure 1.

Usually, the aircraft being refueled are B-47's and KC-97's.

4. Sampling points

As originally planned, samples were to be taken at all points in the system where a change in handling operations occurred as detailed above. In this manner, the effect of that specific operation--whether storing, pumping, screening or filter-coalescing--on the microbial population could be assessed. In addition, for the tank storage conditions, samples were to be taken at three locations in all storage tanks--the water bottom, the interface and the fuel. In discussions with the resident Quartermaster POL Inspector at NETI, it was determined that good housekeeping was practiced at Newington and the bulk fuel storage tanks contained little or no water bottoms. Therefore, it was not possible to obtain fuel, interface and water bottom samples from all of the storage tanks at NETI and Pease Air Force Base. Further, it was not possible to sample the fuel between the pump house and the hose cart at Pease. For these reasons, several planned locations were not sampled. The sampling points for JP-4 and 115/145 Avgas are noted in Figure 1.

5. Sampling techniques

With the aid of a sterile weighted sampler (ASTM D270), 3-gallon composite fuel samples (except for JP-4 sample from Tank #6, NETI) were

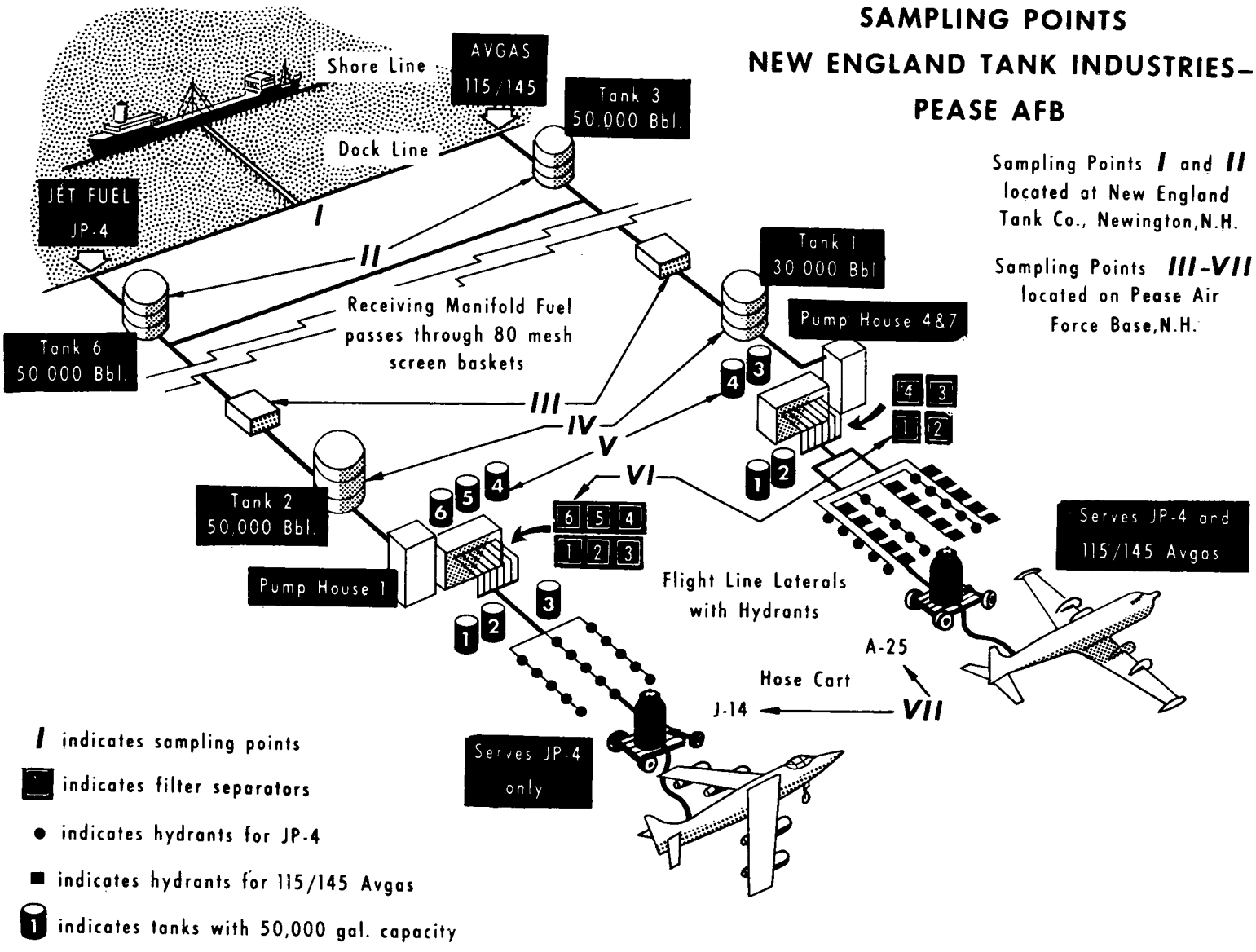

FIGURE 1. Sampling Points - New England Tank Industries - Pease Air Force Base

obtained at each of the storage tank locations shown in Figure 1, by permitting the fuel to enter the sampler as it passed through the lower to upper layers of fuel in the tank. The JP-4 sample taken from Tank #6 at NETI was taken at a point 10 inches from the bottom of the tank to determine if the microbial population was concentrated in the fuel area nearest the water bottom. These were then dispensed in sterile 1-gallon cans. The sampler was rinsed with the fuel, washed thoroughly with a hot detergent solution, rinsed with hot water and then rinsed three times in isopropyl alcohol prior to each use. The hot solutions were approximately 180°F. The water bottom and fuel-water interface samples when present were obtained with the use of a sterile Bacon-type (ASTM D270) sampler and dispensed in 1-quart sterile screw-cap jars.

Line samples and samples from the pump houses, filter separators, and hose carts were obtained by first flushing the drain valve 1 to 2 minutes prior to sampling to remove line contamination and sediment. The fuel samples were collected in sterile 1-gallon cans, and the water fuel samples were collected in sterile, wide-mouth, screw cap 1-quart glass jars.

All samples taken at sampling points I, II and III were under the direct supervision of a trained microbiologist. Since sampling points IV through VII were located within a restricted area, four enlisted personnel from one of the POL crews at Pease Air Force Base were instructed and received demonstrations on the necessity of obtaining samples using the best aseptic techniques. Although it is not certain that samples taken in this restricted area were obtained exercising classical aseptic techniques, the Airman-in-charge reported that all samples were obtained in accordance with the instructions given.

A detailed description of each of the 21 samples obtained is listed in Table I. The physical appearance of Samples 13 through 22 is shown in Figures 2, 3, and 4.

6. Analysis: materials and methods

a. Equipment

To make the analyses in the field, all necessary laboratory equipment and media were transported from Natick to Newington, N. H. Laboratory facilities located at NETI were used to process all samples in the field. All samples were cultured within 1 hour after sampling except for samples 11 and 12 which were held for 16 hours at 40°F prior to culturing (see Table I).

b. Media

The 9 media used in these studies (Appendix A) were selected on the basis of recommendations of the Society for Industrial Microbiology

TABLE I - DESCRIPTION OF FUEL SAMPLES

Location	Sample Number	Type	Date of Sample	Gram Stain of Original Sample	Appearance	Comments
Dock Line (I)* at NETI	3	JP-4 Fuel	22 May		Clear	Fuel had been in the line about 2 weeks
Bulk Storage Tank #6 at NETI 10" above bottom of tank (II)	2	JP-4 Fuel	22 May		Clear	No anti-icing additive in this sample
Bulk Storage Tank #6 at NETI (II)	20	JP-4 Bottom Water-Fuel Sample	24 May	Fungi	Light brown to orange sediment; slight growth at interface	pH 3.5
In-line receiving Manifold at Pease AFB (III)	1	JP-4 Fuel	21 May	Small Gram negative rods	Clear	Contained anti-icing additive
Bulk Storage Tank #2 at Pease AFB (IV) (No water bottom)	9	JP-4 Fuel	22 May		Clear	Contained anti-icing additive
Pump House #1, Tank #5 (V)	5	JP-4 Fuel	22 May		Clear	Contained anti-icing additive and was about 11 days old
Pump House #1 Tank #5 (V)	14	JP-4 Interface	23 May	Fungi	Brown sediment settled to bottom of bottle; medium brown slime at interface	pH 4.5

TABLE I (cont'd)

Location	Sample Number	Type	Date of Sample	Gram Stain of Original Sample	Appearance	Comments
Pump House #1 Tank #5 (V)	13	JP-4 Bottom Water-Fuel Sample	23 May	Many small Gram negative rods, few Gram positive rods, some mold with fruiting bodies (?)	Brown sediment with brown slimy growth adhering to sides of bottle	pH 4.5
Pump House #7, Filter separator #1 (VI)	15	JP-4 Water-Fuel Sample	23 May	Few Gram negative rods, some mold with filaments and fruiting bodies (?)	Large rust particles and brown slimy interface	pH 4.5
Hose Cart J-14 (VII)	7	JP-4 Fuel	22 May		Clear	Contained anti-icing additive
Dock Line (I)	4	115/145 Avgas	22 May		Clear	Fuel had been in line about 3 weeks
Bulk Storage Tank #3 at NETI (II)	10	115/145 Avgas	22 May		Clear	Contained anti-icing additive
Bulk Storage Tank #3 at NETI (II)	21	115/145 Avgas Water Bottom	24 May	Gram negative rods and Gram positive cocci	Orange brown sediment with some brown particles and scum at interface	pH 3.5
In-line receiving Manifold at Pease AFB (III)	11	115/145 Avgas	22 May		Clear	Sample held at 40°F for 16 hrs. prior to analysis
Bulk Storage Tank #1 at Pease AFB (IV)	12	115/145 Avgas	22 May		Clear	Sample held at 40°F for 16 hrs. prior to analysis

TABLE I (cont'd)

Location	Sample Number	Type	Date of Sample	Gram Stain of Original Sample	Appearance	Comments
Pump House #4, Tank 2 (V)	6	115/145 Avgas	22 May		Clear	
Pump House #4, Tank 2 (V)	16	115/145 Avgas Interface	23 May		Brown sediment - very little water present. Fuel: purple and clear	pH 3.5
Pump House #7, Tank 2 (V)	18	115/145 Avgas Water Bottom	23 May		Heavy rusty brown appearance at interface & bottom. Fuel: purple and clear	pH 3.5
Pump House #7, Filter Separator # (?) (VI)	19	115/145 Avgas-Water Bottom	23 May	Gram negative rods	Heavy brown slimy & flaky sediment in water & interface. Fuel: deep purple and clear	pH 4.0
Hose Cart A25 (VII)	8	115/145 Avgas	22 May		Clear	Contained anti-icing additive

*Numbers in parentheses refer to sampling points in Figure 1.

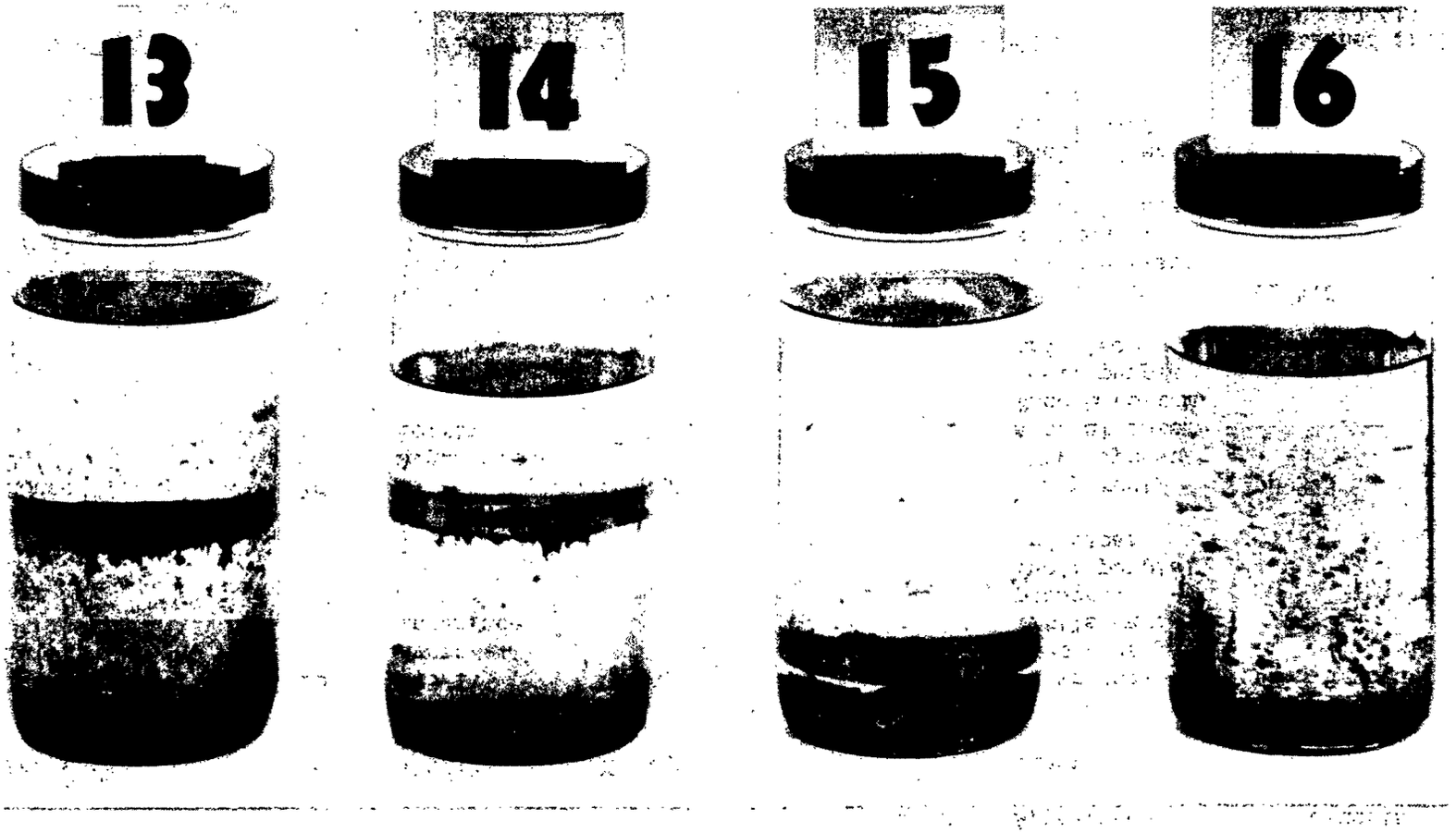

FIGURE 2. Photograph of fuel/water samples 13 through 16. See Table I for detailed description of these samples.

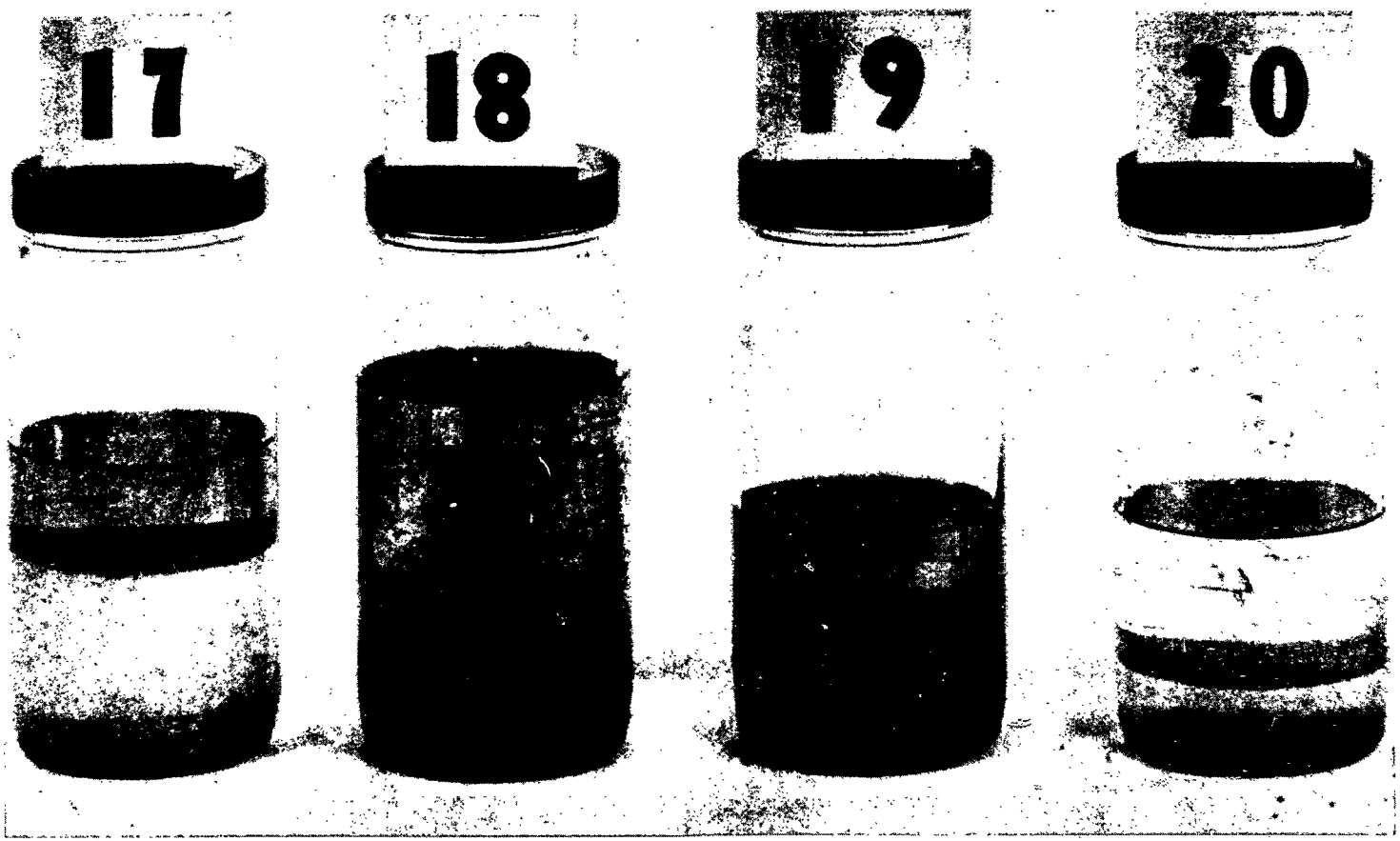

FIGURE 3. Photograph of fuel/water samples 17 through 20. See Table I for detailed description of these samples.

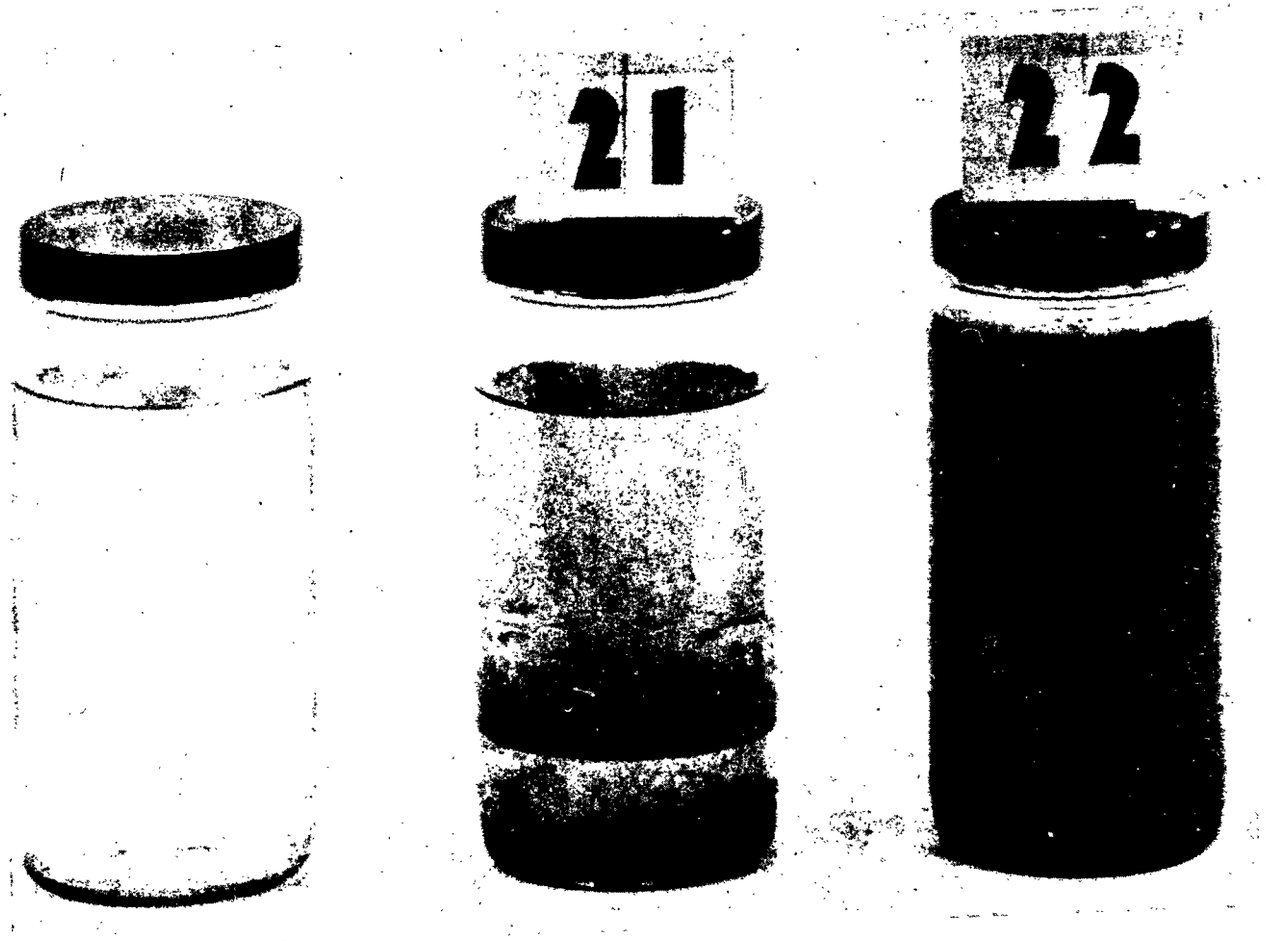

FIGURE 4. Photograph of fuel/water samples 21 and 22. The unlabeled jar on the left is JP-4 fuel obtained from the Dock Line at NETI. See Table I for detailed description of samples.

Committee on Microbiological Deterioration of Fuels (14) and from Dr. Emory Simmons, Pioneering Research Division, for the culturing of fungi.

c. Analysis technique for fuels

Since fuel is not miscible with water or media, it is a difficult material to process for microbial counts by the standard dilution technique. Consequently, the fuel samples were analyzed by the membrane filter technique. This procedure consisted of aseptically filtering 500 ml of fuel through a millipore filter (GS membrane 0.2 μ $\pm$ 0.02 μ poresize obtained from Millipore Filter Corporation, Bedford, Mass.) under reduced pressure. The HA membrane (0.45 μ $\pm$ 0.02 μ poresize), as recommended by the Millipore Filter Corporation and as used in Hazzard's studies (20) will not prevent the passage of certain bacteria. Earlier studies in this laboratory using the HA membrane showed that a Pseudomonas test organism would not be retained by the HA filter. Substituting the finer GS membrane (0.2 μ $\pm$ 0.02 μ poresize) resulted in satisfactory retention of the test organism on this membrane but at a greatly reduced speed of filtration. After the sample was filtered, the funnel and membrane were rinsed with 100 ml of sterile 0.1% alkylaryl polyether alcohol wetting agent (Triton X-100, Rohm & Haas) and then rinsed with 100 ml sterile buffered water (pH 7.2 - see Appendix A). The wetting agent removes the fuel from the membrane and permits migration of soluble medium through the filter when the filter is in contact with agar. After the filter was rinsed, it was placed on top of one of the appropriate agars listed in Appendix A. Care was taken in placing the membrane on the sterile agar to avoid entrapping air bubbles; this permitted direct contact of the filter with the agar. All inoculated filter membranes were incubated at room temperature (21 - 24°C).

All JP-4 and 115/145 Avgas samples were chemically analyzed according to MIL-J-5624 and MIL-G-5572 (see Appendix B).

d. Analysis techniques for interface and water-bottom samples

One ml of the sample was withdrawn with a sterile pipette and delivered into a sterile petri dish to which was added 10 ml of the appropriate medium. In addition, each sample was diluted (ranging between 1 x 10^2 to 1 x 10^6) at regular intervals in sterile buffered water. Each diluted sample was shaken approximately 25 times and appropriate aliquots (0.1 and 1.0 ml) were removed and pipetted into duplicate sterile petri dishes containing about 10 ml of each of the solid media or into the various liquid media listed in Appendix A. The aliquot was thoroughly mixed in the molten agar or liquid media and incubated at room temperature at Newington, N. H., until returned to Natick. At Natick, the plates and flasks were incubated at the temperatures and times specified (Appendix A).

Average counts were made on the number of microorganisms per unit volume of fuel (500 ml) or water (1 ml) obtained with each of the

selective media. In addition, grand average counts per unit volume of fuel or water were then calculated to obtain the relative numbers of bacteria or fungi present per sample by dividing the total count obtained on all the fungal or bacterial media by the number of media used.

7. Results

Tables II and III list the respective microbial counts obtained for all samples. Typical microbial growths are shown in Figures 5 and 6.

a. JP-4 Fuel and Water Samples

Bacteria: Fuel, while in the lines entering NETI (approximately 2 weeks old), contained an average of 4 organisms per 500 ml. As the fuel passed through the distribution system, the concentration remained essentially constant up to the pump house tanks. Fuel leaving the hose cart had increased in count to 55 per 500 ml.

The first bulk storage tank at NETI (Tank #6, 50,000 bbl capacity) always contains at least 1 inch of water bottom. Bacterial counts from the water-bottom sample from this tank averaged approximately 50,000 organisms/ml. Since the next bulk storage tank at Pease Air Force Base (Tank 2, Sampling point IV, Table II) contained no detectible water bottom, no microbial count is reported for this tank. A sample from the 50,000-gallon pump house storage tank (Tank 5, Sample point V, Table II) next to the pump house contained approximately 100 bacteria/ml. However, effluent from the pump house filter separator (point VI, Table II) contained approximately 75,000 bacteria/ml.

Fungi: Fuel entering the dock line (sampling point I) at NETI contained about 2 organisms per 500 ml of fuel. In the first bulk storage tank (sampling point II, Table III), the fungal count rose to 18 per 500 ml of fuel. After this point, the fungal count throughout the system remained consistently low, 3 to 9 organisms per 500 ml. In the water bottom samples, the count was low at the NETI storage and Pease pump house tank, 2 to 7/ml. A significant increase to 114/ml was found in the pump house filter-separator water effluent.

b. 115/145 Avgas Fuel and Water Samples

Bacteria: The bacteria present in the fuel at the dock line were approximately 10 per 500 ml of fuel. As the fuel progressed through the distribution system, the number of bacteria remained essentially constant until the fuel reached the pump house. At the pump house tank, the bacterial count rose to more than 42 per 500 ml, and then dropped to about 4 organisms per 500 ml of fuel at the hose cart outlet. (See Table II)

The bacterial counts in the water bottoms of the storage tanks were low, 150 per ml, dropped significantly at the pump house tank to 3 per ml

TABLE II - RELATIVE NUMBERS OF BACTERIA IN FUEL AND WATER PHASE SAMPLES

Station	Location	Fuel (Counts per 500 ml) Grand Aver.	TGE	API	Water (Counts per ml) Grand Aver.	TGE	API
			JP-4				
I	Dock Line	4	5	2	--- No Water Present ---		
II	First Storage Tank #6, NETI	3	5	1	50,050	100	100,000
III	In-Line	6	11	--	--- No Water Present ---		
IV	Second Storage Tank #2, Pease	7	9	4	--- No Water Present ---		
V	Pump House #1 Tank #5	28	*	28	100	100	100
VI	Pump House #7 Filter Separator #1	Water Sample			75,037	150,000	750
VII	Hose Cart J-14	55	90	19	--- No Water Present ---		
			115/145 Avgas				
I	Dock Line	10	17	2	--- No Water Present ---		
II	First Storage Tank #3, NETI	9	10	8	150	100	200
III	In-Line	9	9	*	--- No Water Present ---		
IV	Second Storage Tank #1, Pease	6	7	5	--- No Water Present ---		
V	Pump House #4 Tank #2	>42	**	42	3	4	2
VI	Pump House #7 Filter Separator #?	Water Sample			150	100	300
VII	Hose Cart A-25	4	3	4	--- No Water Present ---		

TGE=Tryptone Glucose Extract Agar
API=American Petroleum Institute Agar
* =Count Missing
** =TNC (too numerous to count)

TABLE III - RELATIVE NUMBERS OF FUNGI IN FUEL AND WATER PHASE SAMPLES

Station	Location	Fuel (Counts per 500 ml) Grand Aver.	H.D.	Myco.	Czap.	R.B.	PDA	Water (Counts per ml) Grand Aver.	H.D.	Myco.	Czap.	R.B.	PDA
					JP-4								
I	Dock Line	2	0	3	4	3	2	---------- No Water Present ----------					
II	First Storage Tank #6, NETI	18	47	1	37	2	5	2	4	2	2	2	2
III	In-Line	7	4	1	9	9	14	---------- No Water Present ----------					
IV	Second Storage Tank #2, Pease	3	1	3	2	2	8	---------- No Water Present ----------					
V	Pump House #1 Tank #5	9	6	10	3	11	13	7	5	13	0	12	4
VI	Pump House #7 Filter Separator #1	---------- Water Sample ----------						114	50	55	100	213	150
VII	Hose Cart J-14	9	2	2	4	28	7	---------- No Water Present ----------					
					115/145 Avgas								
I	Dock Line	2	2	0	3	1	3	---------- No Water Present ----------					
II	First Storage Tank #3, NETI	9	11	3	9	13	8	1	1	2	0	1	1
III	In-Line	8	7	7	15	2	11	---------- No Water Present ----------					
IV	Second Storage Tank #1, Pease	5	1	7	5	5	7	---------- No Water Present ----------					
V	Pump House#4, Tank#2	4	3	5	3	4	3	1?	2	0	0	0	0
VI	Pump House #7 Filter Separator #?	---------- Mainly Water ----------						1	1	1	1	2	1
VII	Hose Cart A-25	11	6	25	11	7	8	---------- No Water Present ----------					

Note: H.D.=Hay Decoction Agar Myco.=Mycophil Agar Czap.=Czapek Agar R.B.=Rose Bengal PDA=Potato-Dextrose Agar

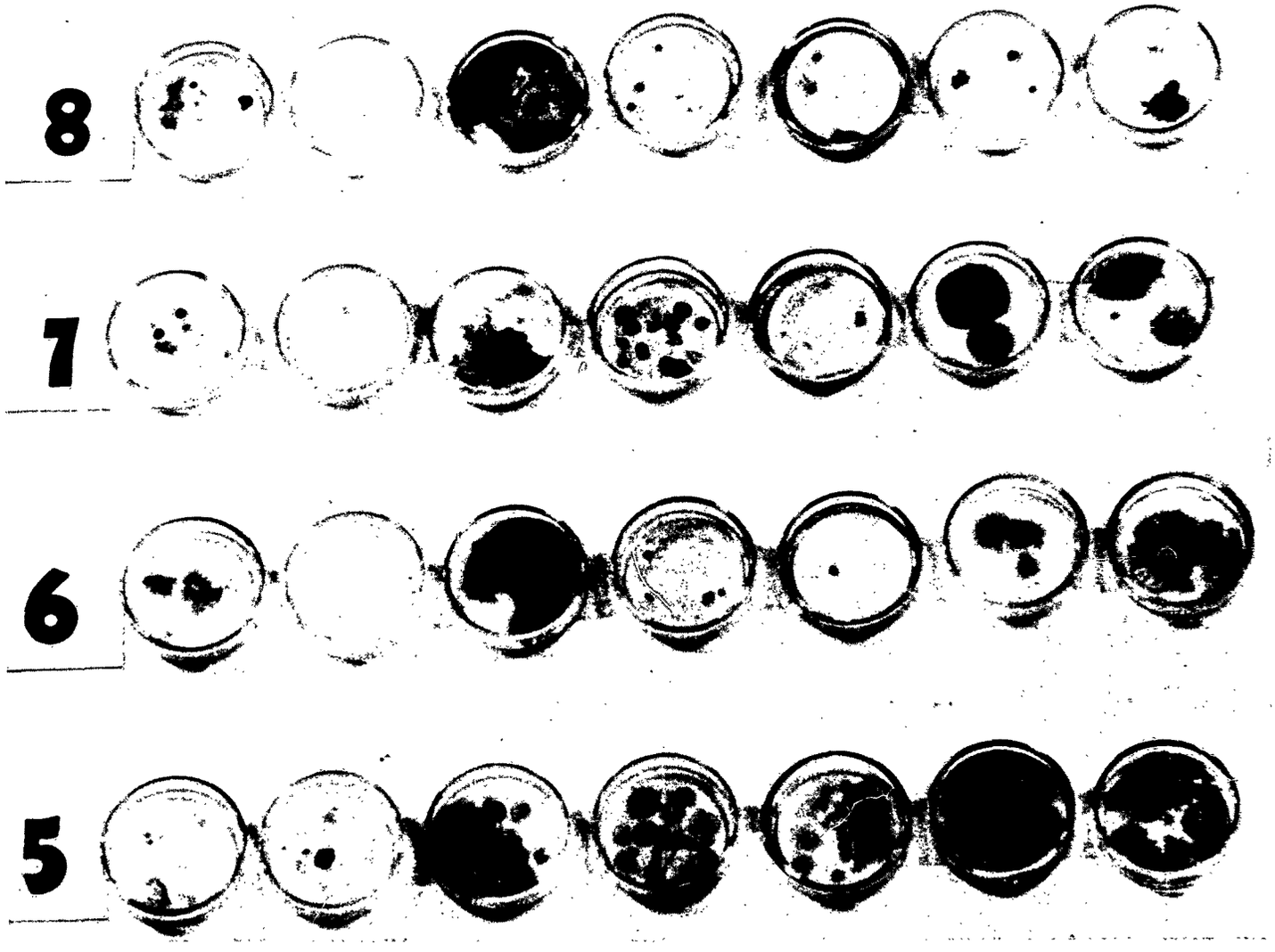

FIGURE 5. Outgrowths on membrane filters of fuel samples 5 through 8 on 7 selective media. From left to right the media are: Tryptone Glucose Agar, American Petroleum Agar, Czapek Agar, Rose Bengal Agar, Hay Decoction Agar, Mycophil Agar, and Potato-Dextrose Agar. A detailed description of samples 5 through 8 is given in Table I.

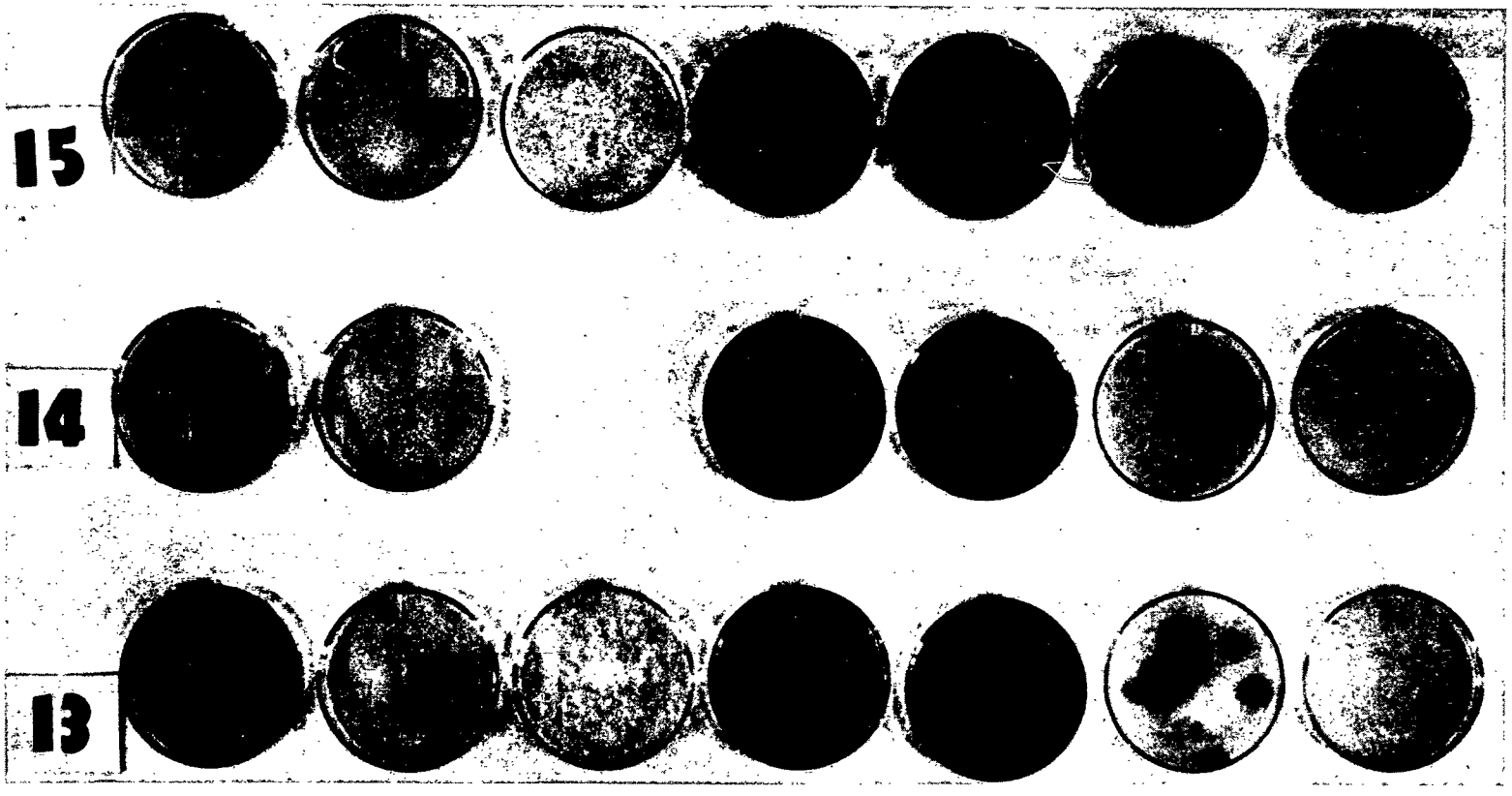

FIGURE 6. Outgrowths on poured plates of water-bottom samples 13, 14 and 15 on 7 selective media. From left to right the media are: Tryptone Glucose Agar, American Petroleum Agar, Czapek Agar, Rose Bengal Agar, Hay Decoction Agar, Mycophil Agar, and Potato-Dextrose Agar. A detailed description of samples 13, 14 and 15 is given in Table I.

and then returned to the original count at the water effluent when the fuel passed through the pump house filter-separator.

Fungi: Fungal counts in the fuel remained quite stable throughout the distribution system, ranging from 2 to 11 per 500 ml from the dock line to the hose cart, respectively.

Fungi in the water samples also remained very stable at approximately 1 per ml.

c. Sulfate-reducing, Sulfur-oxidizing and Iron-depositing Bacteria

No sulfate-reducing or sulfur-oxidizing bacteria were isolated from any of the samples. Gram negative, non-spore forming, iron-depositing bacteria were found in all JP-4 tank water bottoms and filter-separator water effluents. However, these organisms were conspicuously absent in the 115/145 Avgas water bottoms except for the first bulk storage tank at NETI. These organisms were not the classical Sphaerotilus or Gallionella types of iron-depositing bacteria which may have sheaths or deposit iron within the cell.

Since a statistical sampling, such as that required for a most probable number determination, was not made, a quantitative measure of the incidence of these organisms throughout the system could not be determined. It was possible, however, to note a relative increase in the presence of these organisms at the water effluent from the pump house filter-separators for JP-4, as contrasted with the rest of the system, since various dilutions of the water samples were inoculated into the liquid media and growth was obtained at 10^{-7} dilutions at this point versus 10^{-2} to 10^{-4} dilutions for other points.

Figure 7 illustrates the growth of these bacteria in the iron medium.

d. Dominant types of bacteria and fungi isolated from water-bottom and fuel samples

Four general types of bacteria were isolated from the fuel-water samples. The following types of organisms are listed in the order of decreasing prevalence:

1. Gram negative iron-depositing bacteria
2. Gram negative small rods
3. Gram positive cocci
4. Gram positive medium and large rods

As time permits, the bacteria isolated will be characterized in greater detail.

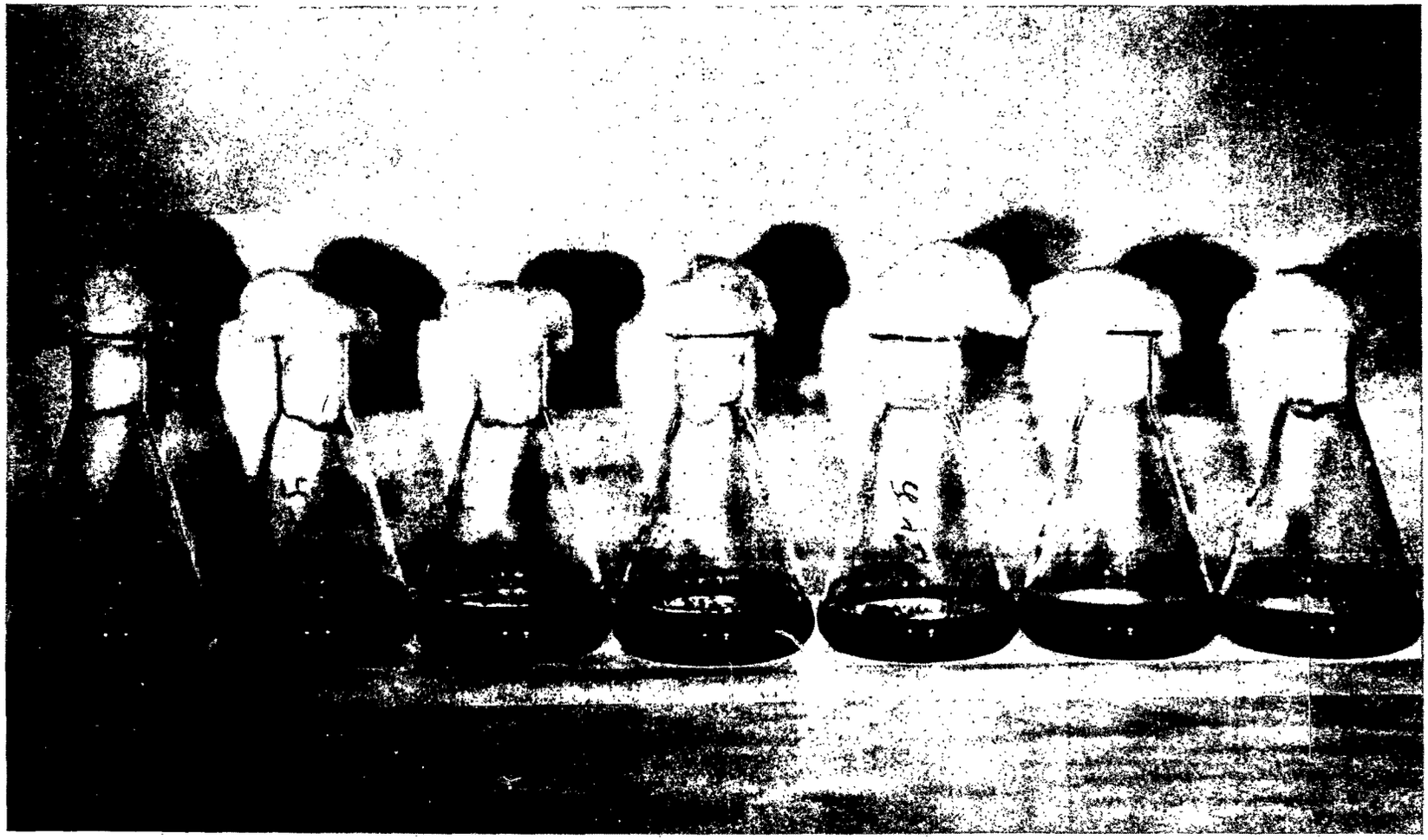

FIGURE 7. Typical growth of the Gram negative iron deposition bacterium isolated in water-bottom samples. Flasks on extreme right are the uninoculated control for comparison.

Over 800 fungal cultures were obtained for study from water and fuel samples. These isolates are being studied with selections of different types being made. After elimination of duplicates and purification, a determination will be made as to which are purely adventitious and which will grow on hydrocarbon fuels. A high percentage of the isolates are species of Cladosporium. Tentatively identified are forms belonging to the genera Fusarium, Alternaria, Cephalosporium, Paecilomyces, Oidium, Scopulariopsis and Pullularia as well as several black, orange, and white yeasts and actinomycetes.

e. Microbial Population of a Filter-Separator

During the field study of 21 to 25 May 1962, it was not possible to examine a filter-separator cartridge. On 14 June 1962, however, a cartridge, designated FSM 4930-774-2213, Cartridge, Coalescer, Part No. CC-K2, was removed from the JP-4 line at 1100 hours at Pease Air Force Base and examined at 1400 hours at the Natick Laboratories. The cartridge was removed after a 2,000,000 gallon throughput. The cartridge at this time was still functional and appeared clean to visual examination.

The stocking and metal cover were cut through with sterile tin-snips to expose the paper and glass fiber filter element. Then 1 x 1 inch strips of the filter were cut and removed aseptically and cultured on the media previously discussed. Figure 8 shows some of the microbial growth obtained from the filter. Six Gram positive bacilli, one Gram negative rod and six fungi were isolated, but no attempt was made to quantify the number of microorganisms present per filter area.

f. Chemical Analysis

All fuel samples passed the specification chemical tests as noted in detail in Appendix B.

8. Discussion

a. Microbial Population

This study confirms the fact that JP-4 and Avgas 115/145 in a military supply system do contain microorganisms, that the microorganisms are found throughout the distribution system, that the filter-coalescers fail to remove microorganisms from the fuel.

The fuel microbial counts obtained are low and may be representative only of a system where good petroleum housekeeping is practiced. There appears to be no significant difference in the order of magnitude of fungal and bacterial fuel counts between JP-4 and 115/145 Avgas. This also holds true for the fungal counts for the water samples from both JP-4 and 115/145 Avgas systems. For the bacterial counts, however, a significantly greater concentration was found for water samples from the

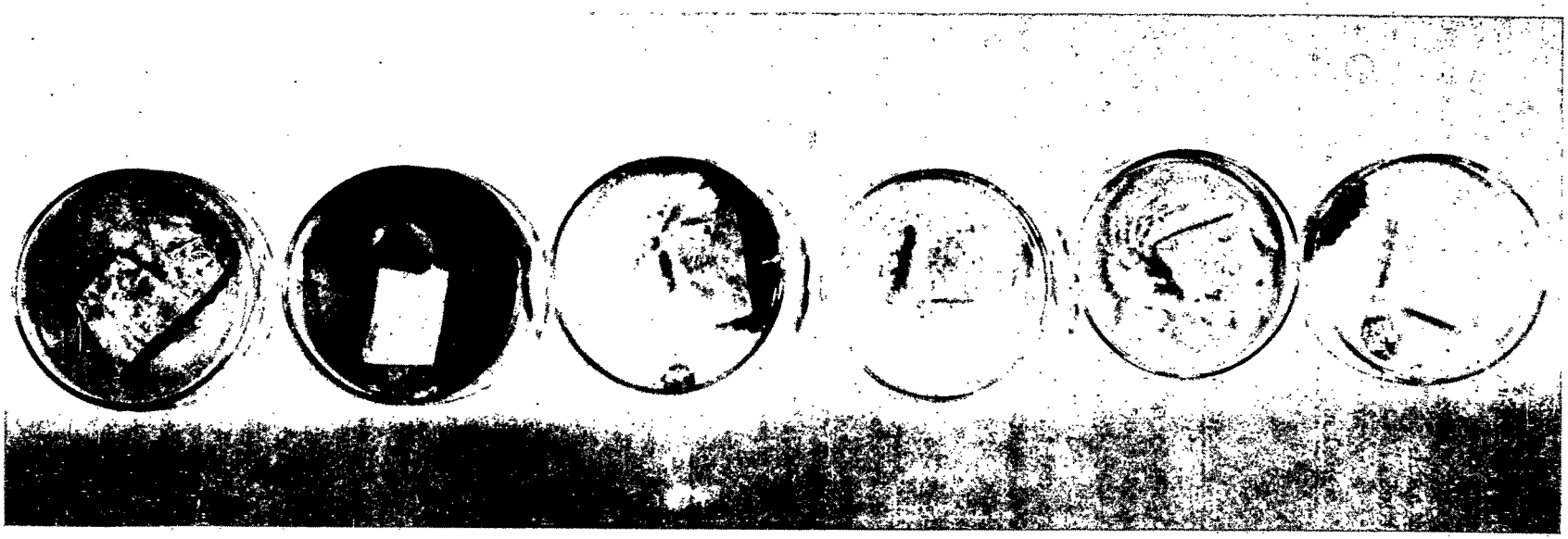

FIGURE 8. Typical bacterial and fungal outgrowths from a JP-4 fuel filter cartridge coalescer.

JP-4 system than for those from the Avgas system. It is interesting to note that where a high bacterial count was observed in the water bottom of the first JP-4 storage tank at NETI, approximately 50,000/ml, a corresponding high count was not observed in the fuel from this tank.

In general, there was no particular sampling station in the system that showed significant buildup of microbial counts for both JP-4 and 115/145 Avgas fuels. Certain stations showed an increase in absolute count but the increase is not considered to be microbiologically significant when the counts are of as low order of magnitude as observed in these studies. For example, the increase in bacterial count to an average of 28 per 500 ml and 55 per 500 ml observed for JP-4 fuel at the pump house tank and at the hose cart effluent, respectively, as compared to an average of 3 to 7 per 500 ml in the previous stations, does not appear to be serious. The tendency of counts to increase at these points, however, may take on significance if the fuel counts are high and if it is established that these points are foci for buildup of counts due to their particular environment. Under these conditions, these points would serve as loci of infection, and remedial action would be required to remove this source of system contamination. Indeed, a case can be made for the filter cartridge acting as a prime source of contamination. As reported by Knecht and Watkins for fuel oils (18), a filter element provides an ideal surface for microbial growth and activity. Essential nutrients such as water, minerals and organic matter are carried to and concentrated in the element. Oxygen is available. Soluble waste products that would normally accumulate and tend to inhibit growth are carried away as the fuel passes through. Thus an ideal dynamic culture system is set up. No clear-cut evidence is available, however, to demonstrate that this is the reason for the increased count at the filter-separator. The increase in absolute count could equally as well be attributed to agitation of the fuel stream and better distribution of microorganisms in the sample to give an apparent increase. The important point, however, is that the tendency for an increased count to occur at these points should be kept in mind, particularly if the fuel stream shows high counts prior to reaching these stations.

The variation in bacterial counts of water samples from JP-4 and 115/145 Avgas systems from an initial level at the first storage tank at NETI to a drop of 2 logs per ml in count at the pump house storage tank and then an increase in count back to the original level at the pump house filter-separator water effluent is significant, as is the difference in count between JP-4 and 115/145 Avgas systems water. The higher count at the NETI tanks for the JP-4 vs. the 115/145 Avgas, 50,000 per ml vs. 150 per ml, may be due to the fact that JP-4 appears to more readily support growth than the Avgas in the Laboratory. It is not due to pH differences, since there was no apparent relationship between the pH of the water phase and microbial populations. More probably, the difference may be due to the fact that there was always 1 inch of water in the JP-4 tank, which would permit organisms to increase over a given time period; whereas the water in the Avgas tank could be drained "completely", thereby preventing

the growth of microorganisms by periodical removal of the water environment and the water contaminants. This could also explain the drop in count at the pump house tank where the water was drained daily. The higher levels in the water at the filter-separator effluent could represent washing out of bacteria that were concentrated on the surface of the filter cartridge. The foregoing explanations are conjecture at this point since these specific studies were not set up to determine the basic reasons for microbial changes in the system. Indeed, concern with counts in the water bottom may be academic as contrasted with fuel counts since the high water sample counts in these studies were not correlated with high counts in the fuel. This is not to say that water counts can be completely disregarded since, again, very high counts may indicate potential trouble spots.

b. Media

In these studies, five different media were used to count fungi and two different media were used for bacterial counts. No definite recommendations as to which media are superior can be made at this point. The media were chosen for their selectivity for different types of fungi, and studies as of this date have not progressed far enough to determine whether the counts with each of the different media represent different genera or if similar organisms appeared on all the media. Indeed, fungal counts, although expressed quantitatively, should be considered to be only qualitative. This is because a single fungal hypha may be fragmented during culturing procedures, giving rise to a number of fungal colonies from the fragments and thereby indicating a count higher than the true count. Or conversely, one mycelial mass may have formed from many spores but would only be counted as one colony.

For the bacteria, the tryptone glucose extract medium generally appears to be superior to the API medium for fuel sampling. For water sampling, no choice can be made.

The presence of iron depositing bacteria in almost all the JP-4 water samples is considered significant since their presence indicates a presumptive potential for microbially induced storage tank and pipe line corrosion and plugging. The failure to obtain any evidence of sulfate reducers or oxidizers may truly indicate their absence from the system. There is always doubt, however, as to the adequacy of culturing techniques now employed for the isolation of these organisms.

c. Significance of Counts

The significance of the counts obtained in this survey is not known. It is not possible to say, for example, whether a concentration of 50 bacteria/500 ml of fuel is indicative of a potential problem. The counts must be correlated with problems such as filter-plugging, wing-tank corrosion, failure of fuels to pass specification fuel consumption, and other

tests, and excessive corrosion of pipe lines, tanks, and fuel handling equipment. None of these problems has been reported by Pease Air Force Base. Microbial counts at best can only be considered a tool for appraising the effectiveness of the petroleum handling system. If fuels contain large numbers of organisms, it is fairly safe to assume that the best handling and storage procedures are not being practiced, or possibly the fuel had undergone extensive storage. Unless remedial action is taken to lower the count by any one of a number of corrective procedures, the risk of bringing about a potential fuel problem becomes imminent. If fuel handling practices at Pease Air Force Base are considered to be representative of good housekeeping, there is no question that these practices insure low-count fuel. It should be noted, however, that probably no practical good housekeeping system can be depended upon to provide uncontaminated fuel.

When sufficient comparable data on the concentration of microorganisms present in fuels become available from a number of installations, including bases that have a high incidence of fuel problems, it may be possible to correlate such data with the degree or seriousness of the contamination. The results of routine microbiological examinations of fuel samples cannot be considered as providing complete or final information on the quality of the fuel. The results must be considered in the light of the fuel handling practices and careful consideration must be given to all the relevant factors, including experience and frequency of fuel problems, before any finite count can be set as a quality standard for fuel distribution systems.

9. Acknowledgements

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APPENDIX A

MEDIA FOR MICROBIOLOGICAL EXAMINATION OF FUELS

Media For Enumerating Bacteria

1. Tryptone Glucose Extract Agar (TGE)

To each liter of distilled water add 3 g beef extract, 5 g tryptone, 1 g glucose, and 15 g agar. Adjust pH so that after sterilization at 15 lbs pressure (121°C) for 15 minutes, the final pH will be between 6.8 and 7.0.

2. Medium for Isolating and Counting Sulfate Reducing Bacteria (API RP-38)

Sodium Lactate, USP	4.0	ml
Yeast Extract	1.0	g
Ascorbic Acid	0.1	g
Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)	0.2	g
Potassium Phosphate, Dibasic (K_2HPO_4, anhydrous)	0.01	g
Ferrous Ammonium Sulfate ($Fe(SO_4)_2 (NH_4)_2 \cdot 6H_2O$)	0.1	g
Sodium Chloride (NaCl)	10.0	g
Agar	15.0	g
Distilled Water	1000.0	ml

The ingredients should be dissolved with gentle heating. The pH should then be adjusted to 7.5 with NaOH. If excessive precipitation occurs, the medium should be discarded. The medium is dispensed into test tubes (9 ml per tube), which are then autoclaved for 10 minutes at 15 psi steam pressure. Screw caps or rubber stoppers should be used to seal the tubes to prevent dehydration of the medium. Remelting agar more than once is not recommended.

3. Medium for Sulfur-Oxidizing Bacteria (Waksman-19)

Ammonium Sulfate ($(NH_4)_2SO_4$)	0.2	g
Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)	0.5	g
Calcium Chloride ($CaCl_2$)	0.25	g
Potassium Phosphate, Monobasic (KH_2PO_4)	3.0	g
Distilled Water	1000.0	ml

Dispense in 100 ml aliquots in 250 ml Erlenmeyer flasks. Add 1.0 g elemental sulfur to each flask. Steam sterilize at 100°C for 30 minutes for 3 consecutive days. Inoculate with 10 ml aliquots of each water-fuel sample and incubate at 25°C for 60 days. Growth of sulfur-oxidizing

bacteria is indicated by turbidity and a decrease in the pH of the media as a result of the formation of sulfuric acid. To confirm the absence of such bacteria, portions from each flask should be streaked on Waksman's thiosulfate agar plates. The plates should be incubated at 25°C for 2 weeks.

4. Thiosulfate Agar (Waksman-19)

Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)	5.0	g
Ammonium Chloride (NH_4Cl)	0.1	g
Sodium Bicarbonate ($NaHCO_3$)	1.0	g
Sodium Phosphate, Dibasic ($Na_2HPO_4 \cdot 2H_2O$)	0.2	g
Magnesium Chloride ($MgCl_2 \cdot 6H_2O$)	0.1	g
Agar	15.0	g
Tap Water	1000.0	ml

Sterilize the thiosulphate and acid carbonate in a small amount of water and, when cool, add to the solution of the other salts and agar. A trace of ferrous sulphate (sterile solution) should be added after sterilization.

5. Medium for Iron-Depositing Bacteria (Waksman-19)

Ammonium Sulfate ($(NH_4)_2SO_4$)	0.5	g
Sodium Nitrate ($NaNO_3$)	0.5	g
Dipotassium Phosphate (K_2HPO_4)	0.5	g
Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)	0.5	g
Calcium Chloride ($CaCl_2$)	0.2	g
Ferric Ammonium Citrate	10.0	g
Distilled Water	1000.0	ml

Dispense 100 ml in 250 ml Erlenmeyer flasks and sterilize at 121°C for 15 minutes. Sterilize ferric ammonium citrate separately and add when cool.

Solid medium is prepared by the addition of 2.0% agar.

Inoculate with 10 ml portions of the fuel-water sample. Incubate at 25°C for 45 days. During and at the end of the incubation period, agar plates of the same medium are streaked with portions from each flask and incubated at 25°C for two weeks.

Media for Enumerating Molds and Yeasts

6. Hay Decoction Agar (Designed as an isolation medium which permits early sporulation with minimal mycelial production).

Decomposing Hay (roadside grasses)	50.0	g
Distilled Water	1000.0	ml

Chop hay into approximately 1-inch pieces. Autoclave for 30 minutes at 15 lbs. Filter.

Filtrate	1000.0	ml
Potassium Phosphate, Dibasic (K_2HPO_4)	2.0	g
Agar	20.0	g

Autoclave 10 minutes. Adjust to pH 6.0 to 6.5. Sterilize at 15 lbs for 20 minutes. Inoculate with 1.0 ml portions of water-fuel sample and incubate at 25-26°C until growth appears.

7. Mycophil Agar (Designed for organisms requiring low pH)

Phytone	10.0	g
Dextrose	10.0	g
Agar	18-20	g
Distilled Water	1000.0	ml

Adjust to final pH 4.7 by adding 15 ml of sterile 10% lactic acid to each liter prior to plating but after medium has cooled to 55°C. Do not reheat after acidification.

Inoculate with 1.0 ml portions of water-fuel sample and incubate at 25-26°C.

8. Czapek Agar plus Yeast Extract (Specially advantageous for the isolation of Penicillia and Aspergilli)

Sodium Nitrate ($NaNO_3$)	3.0	g
Potassium Phosphate, Dibasic (K_2HPO_4)	1.0	g
Yeast Extract	0.5	g
Magnesium Sulfate ($MgSO_4$)	0.5	g
Potassium Chloride (KCl)	0.5	g
Ferrous Sulfate ($FeSO_4$)	0.01	g
Sucrose	30.0	g
Distilled Water	1000.0	ml

Dissolve all ingredients except sugar in water; potassium phosphate should be added last, after all other salts have been dissolved. Autoclave 10 minutes. Add sugar and autoclave at 15 lbs for 20 minutes.

Inoculate with 1.0 ml portions of water-fuel sample and incubate at 25-26°C.

9. Rose Bengal - Streptomycin Agar (Useful in controlling bacteria and permitting fungal growth on mixed plates).

Dextrose	10.0	g
Peptone	2.0	g
Potassium Phosphate, Dibasic (K_2HPO_4)	0.5	g
Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)	0.5	g
Agar	15.0	g
Distilled Water	1000.0	ml
Rose Bengal	50.0	mg
Streptomycin	8.0	ml of 1% aqueous solution

Dissolve all ingredients except streptomycin in water. Heat with occasional agitation and boil for about 1 minute. Dispense and autoclave at 116-118°C (10-12 lbs) for 15 minutes. The streptomycin solution is sterilized by filtration through a Seitz or other filter and the appropriate amount added to the basic medium after it has been melted and then cooled sufficiently for pouring plates (less than 55°C).

Inoculate with 1.0 ml portions of water-fuel mixture sample and incubate at 25-26°C.

10. Potato-Dextrose Agar (Useful for certain organisms that won't grow well on the other media specified).

Potato Extract	100.0	ml
Dextrose	5.0	g
Agar	20.0	g
Distilled Water	900.0	ml

Autoclave at 15 lbs for 20 minutes.

Final pH 5.6.

Buffered Dilution Water

Stock Phosphate Buffer Solution: Dissolve 34.0 g potassium dihydrogen phosphate, KH_2PO_4, in 500 ml distilled water, adjust to pH 7.2 with 1 M NaOH and made up to 1 liter with distilled water. Add 1.25 ml stock phosphate buffer solution to 1 liter distilled water. Dispense in amounts that will provide 99 $\pm$ 2 ml, or 9 ml $\pm$ 0.02 ml, after autoclaving at 15 lbs for 20 minutes.

APPENDIX B

CHEMICAL ANALYSIS OF SAMPLES OF 115/145 AVGAS & JP-4

Obtained from New England Tank Co. and Pease Air Force Base

Analysis by G. Calef

Sample identification: Avgas 115/145

Samples obtained from Pease Air Force Base, Portsmouth, N. H.*

Sample Number		
4	I	Dock Line Sample, NETI
6	VI	Pump House #4 - Tank 2, Pease
8	VII	Hose Cart A-25, Pease
10	II	First Storage Tank #3 - NETI
11	III	In line, Pease
12	IV	Second Storage Tank #1, Pease

*Sampling points are given in Figure 1.

Avgas 115/145 - Portsmouth

Specification Test	Specification Requirement	Sample Number 4	6	8	10	11	12
Gravity-Degrees, API		69.4	69.1	69.1	69.3	69.4	69.4
Color (Visual)		Purple	Purple	Purple	Purple	Purple	Purple
Appearance		Clear	Clear	Clear	Clear	Clear	Clear
Distillation-Degrees F							
Initial Boiling Point		120	118	102	102	109	109
End Point	338 Max.	332	313	333	324	335	326
10% Evaporated	@167 Min.	145	145	143	135	145	144
40% Evaporated	@167 Max.	196	195	200	194	197	196
50% Evaporated	@221 Min.	210	207	211	209	210	209
90% Evaporated	@221 Min.	237	238	239	238	237	238
Residue, Vol. %	1.5 Max.	0.8	0.7	0.8	0.7	1.0	0.7
Distillation Loss %	1.5 Max.	1.5	1.5	1.2	1.5	0.3	0.8
Existent Gum mg/100ml	3.0 Max.	0.4	1.2	0.4	1.4	1.0	0.6
Potential Gum mg/100 ml	6.0 Max.	5.2	1.6	5.3	3.4	3.0	1.6
Sulfur - Total-%	0.05Max.	0.02	0.019	0.014	0.018	0.018	0.029
Reid Vap.Pres-PSI	5.5 - 7.0	6.57	6.37	6.15	6.43	6.50	6.59
T.E.L. ml/gal.	4.6 Max.	4.25	3.95	4.36	4.14	4.34	4.43
Aniline Point Degrees F.		150.6	149.5	150.3	150.3	150.6	150.3
Aniline-Gravity Product	10,000 Min.	10,430	10,320	10,380	10,420	10,450	10,430
B.T.U/lb Net	18,900 Min.	18,956	18,946	18,956	18,955	18,958	18,955
Potential Gum-Precipitate-mg/100ml	2.0 Max.	0.1	0.1	1.8	0.4	0.00	0.2
Potential Gum N.Heptane Wash		2.2	1.0	1.2	0.3	1.3	1.3
Water Reaction Vol. Change ml.	2.0 Max.	Nil	Nil	Nil	Nil	Nil	Nil
Copper Strip Corrosion, ASTM-Classification	1 Max.	1A	1A	1A	1A	1A	1A
Freezing Point Degrees F.	Max. Minus 76	All samples remaining in liquid phase to Minus 100 F.					

SAMPLE IDENTIFICATION - JP-4

Obtained from Pease Air Force Base, Portsmouth, N. H.

SAMPLE		
1	III	In Line, Pease
2	II	First Storage Tank #6, NETI
3	I	Dock Line
5	V	Pump House #1, Tank #5
7	VII	Hose Cart J-14
9	IV	Second Storage Tank #2, Pease

JP-4 - Pease Air Force Base, Portsmouth, N. H.

Specification Test	Specification Requirement	Sample Number 1	2	3	5	7	9
Gravity - Degrees API	45-57	54.7	51.4	54.6	54.5	54.1	54.6
Color (Visual)		White	White	White	White	White	White
Appearance		Clear	Clear	Clear	Clear	Clear	Clear
Distillation °F.							
Initial Boiling Pt.		158	128	175	172	165	172
End Point		395	468	415	426	422	413
10% Evaporated		227	212	233	227	227	229
20% Evaporated	@290 Min.	247	263	250	243	245	247
50% Evaporated	@370 Min.	269	321	272	269	273	272
90% Evaporated	@470 Min.	321	408	326	333	358	327
% Evap. @ 400°F			86.5	97.0	95.5	94.0	96
Residue ml	1.5 Max.	0.9	1.1	0.8	0.8	0.9	0.9
Loss Vol. %	1.5 Max.	1.4	1.4	1.2	1.5	1.1	1.5
Existent Gum mg/100 ml	7.0 Max.	0.4	0.2	0.6	0.4	0.6	2.4
Potential Gum mg/100ml	14.0 Max.	4.4	5.6	4.0	4.0	7.6	4.2
Sulfur-Total-%	0.4 Max.	0.044	0.033	0.058	0.061	0.044	0.044
Reid Vap.Pres.PSI	2.0 - 3.0	2.23	2.22	2.30	2.10	2.18	2.27
Aromatics - Vol.%	25.0 Max.	14.7	13.6	13.5	14.0	14.6	14.0
Olefins - Vol.%	5.0 Max.	0.29	0.88	0.45	0.53	0.42	0.47
Copper Strip Corrosion	1 Max.	1A	1A	1A	1A	1A	1A
Water Reaction Max.Vol.Change	1.0 ml	Nil	Nil	Nil	Nil	Nil	Nil
Methyl cellosolol-glycerin %	0.09-0.15%	0.109	0.00	0.11	0.078	0.057	0.091
Bromide No.		0.4	1.05	0.61	0.72	0.57	0.64
Aromatics + Olefins Vol. %		15.0	14.5	14.0	14.5	15.0	14.5
Aniline Point °F.		124.9	133.5	125.8	127.4	127.0	126.0
Aniline-Gravity Prod.	5,250 Min.	6840	6860	6860	6950	6870	6870

JP-4 - Pease Air Force Base, Portsmouth, N. H.

Specification Test	Specification Requirement	Sample Number 1	2	3	5	7	9
Precipitate - from Potential Gum-mg/100ml		0.00	0.6	0.4	2.4	0.00	0.00
N.Heptane Wash Potential Gum		0.7	1.1	0.4	0.8	4.6	3.4
B.T.U/lb	18,400 Min.	18,693	18,696	18,693	18,698	18,696	18,696
Mercaptan-sulfur % wt	0.001 Max	Nil	Nil	Nil	Nil	Nil	Nil
Smoke Point mm		22	24	20	24	25	25
Smoke Volatility Index	52.0 Min.	64	60.4	60.7	64.2	64.5	65.3
Sediment from millipore filtration-mg/gal.	See Note	2.4	4.3	1.9	0.95	5.7	0.00
Moisture - H_2O ppm Vol. basis		43.5	32.6	36.6	34.6	31.1	34.6
Freezing point Degree F.	Max. Minus 76	All samples remained in liquid phase down to minus 100°F.					

NOTE: Sediment from millipore filtration T.O. 42 B1-1-13 specifies: If non-combustible solids content of fuel exceeds 8 mg/gal - system should be shut down. The data listed above include both combustible and non-combustible sediment.

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